Validation & Assay Performance Summary

GeneBLAzer® PPAR gamma DA Cells & Assay Kit

GeneBLAzer® PPAR gamma UAS-*bla* HEK 293H Cells

Cat. no. K1419, K1701

Target Description

Peroxisome Proliferator-Activated Receptor-gamma (PPAR gamma) is a member of the nuclear receptor family of ligand-activated transcription factors that heterodimerize with retinoic acid-like receptor (RXR). It is involved in the regulation of glucose and lipid metabolism. Agonists include the class of anti-diabetic agents called thiazolidinediones (TZD) or "glitazones." Rosiglitazone (Avandia) and Pioglitazone (Actos) are two PPAR gamma agonists currently marketed for treatment of type 2 diabetes. PPAR gamma agonists may also be therapeutically important in the treatment of coronary artery disease, obesity, and cancer.

Cell Line Description

GeneBLAzer® PPAR gamma DA (Division Arrested) cells and PPAR gamma-UAS-*bla* HEK 293H cells contain the ligand-binding domain (LBD) of the human Peroxisome Proliferator-Activated Receptor-gamma (PPAR gamma) fused to the DNA-binding domain of GAL4 stably integrated in the GeneBLAzer® UAS-*bla* HEK 293H cell line. GeneBLAzer® UAS-*bla* HEK 293H cells stably express a beta-lactamase reporter gene under the transcriptional control of an upstream activator sequence (UAS). When an agonist binds to the LBD of the GAL4 (DBD)-PPAR gamma (LBD) fusion protein, the protein binds to the UAS, resulting in expression of beta-lactamase. Division Arrested (DA) cells are available in two configurations- an Assay Kit (which includes cells and sufficient substrate to analyze 1 x 384-well plate), and a tube of cells sufficient to analyze 10 x 384-well plates.

DA cells are irreversibly division arrested using a low-dose treatment of Mitomycin-C, and have no apparent toxicity or change in cellular signal transduction. Both PPAR gamma DA cells and PPAR gamma-UAS-*bla* HEK 293H cells are functionally validated for Z' and EC_{50} concentrations of Rosiglitazone (Figure 1). In addition, PPAR gamma-UAS-*bla* HEK 293H cells have been tested for assay performance under variable conditions, including DMSO concentration, cell number, and stimulation time.

Validation Summary

Performance of this assay was validated under optimized conditions in 384-well format using LiveBLAzer™-FRET B/G Substrate.

1. **Primary agonist dose response under optimized conditions (n=6)**

	DA	Dividing
Rosiglitazone EC_{50}	5.5 nM	3.7 nM
Z'-Factor (EC_{100})	0.93	0.95

Response Ratio = 5
Optimum cell no. = 30K cells/well
Optimum [DMSO] = up to 1%
Stimulation Time = 16 hours
Max. [Stimulation] = 300 nM

2. **Alternate agonist dose response**
   See agonist dose response section

3. **Antagonist dose response**
   See antagonist dose response section

4. **Cell culture and maintenance**
   See Cell Culture and Maintenance Section and Table 1

Assay Testing Summary

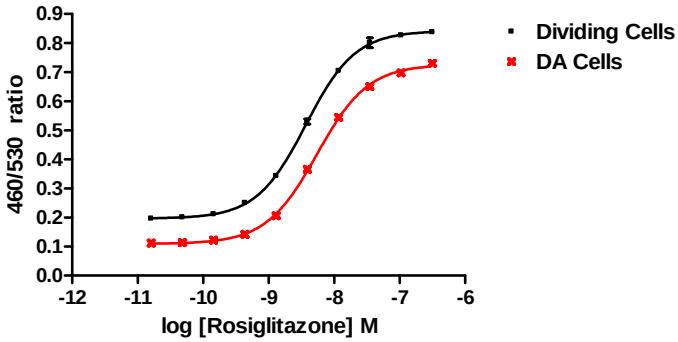
5. **Assay performance with variable cell number**

6. **Assay performance with variable DMSO concentration**

7. **Assay performance with variable stimulation time**

Primary Agonist Dose Response

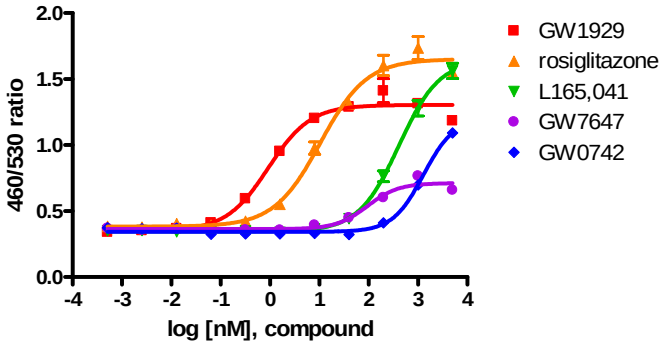
Figure 1 — PPAR gamma DA and PPAR gamma-UAS-*bla* HEK 293H dose response to Rosiglitazone under optimized conditions



PPAR gamma DA cells and PPAR gamma-UAS-*bla* HEK 293H cells (30,000 cells/well) were plated in a 384-well format and serum starved for 24 hrs. Cells were then stimulated with a dilution series of Rosiglitazone in the presence of 0.5% DMSO for 16 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate (1µM final concentration of CCF4-AM) for 2 hours. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and plotted for each replicate against the concentrations of Rosiglitazone (n=6 for each data point).

Alternate Agonist Dose Response

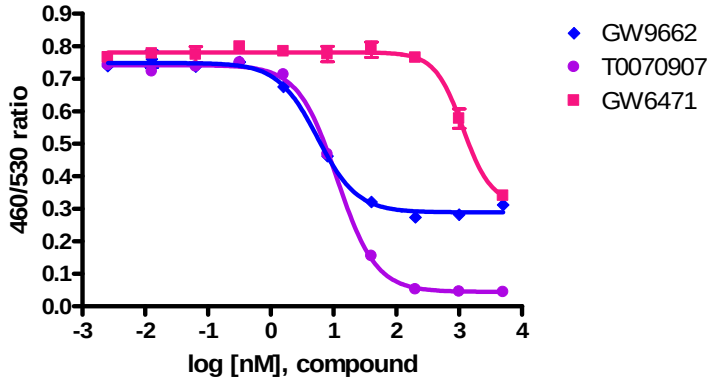
Figure 2 — PPAR gamma-UAS-*bla* HEK 293H dose response to alternate agonists



PPAR gamma-UAS-*bla* HEK 293H cells were serum starved for 24 hours and then plated (30,000 cells/well) in a 384-well black-walled poly-D-lysine assay plate. Cells were stimulated with either GW1929, Rosiglitazone, L165,041, GW7647, or GW0742 over the indicated concentration range in the presence of 0.5% DMSO for 16 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate (1µM final concentration of CCF4-AM) for 120 minutes. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the Ratios plotted against the indicated concentrations of the agonists.

Antagonist Dose Response

Figure 3 — PPAR gamma-UAS-*bla* HEK 293H dose response to known antagonists



PPAR gamma-UAS-*bla* HEK 293H cells were serum starved for 24 hours and then plated (30,000 cells/well) in a 384-well black-walled poly-D-lysine coated assay plate. Cells were treated with antagonists GW9662, T0070907, and GW6471 and incubated at 37 degrees C for 30 min., followed by 47 nM Rosiglitazone agonist stimulation for 16 hours in 0.5% DMSO. Cells were then loaded for 120 minutes with LiveBLAzer™-FRET B/G Substrate. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the Ratios are shown plotted against the indicated concentrations of ligand.

Dividing Cell Culture and Maintenance

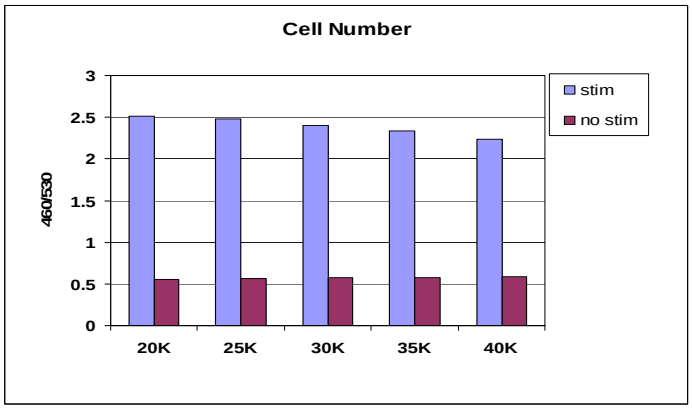
Dividing cells should be maintained at between 5 and 90% confluency in complete growth media and in a humidified incubator at 37ºC and 5% CO_2. Split dividing cells at least twice a week. Do not allow dividing cells to reach confluence. Cells must be grown on Matrigel-coated flasks.

Table 1 – Dividing Cell Culture and Maintenance

Component	Growth Medium (–)	Growth Medium (+)	Assay Medium	1X Matrigel Matrix	Freeze Medium
DMEM, w/ GlutaMAX™	90%	90%	—	99.75%	—
DMEM, phenol red-free	—	—	99%	—	—
Dialyzed FBS **Do not substitute!**	10%	10%	—	—	—
FBS, charcoal-dextran stripped	—	—	1%	—	—
Matrigel™ Matrix	—	—	—	0.25%	—
NEAA	0.1 mM	0.1 mM	—	—	—
Sodium Pyruvate	1 mM	1 mM	—	—	—
HEPES (pH 7.3)	25 mM	25 mM	—	—	—
Hygromycin	—	100 µg/mL	—	—	—
Penicillin	100 U/mL	100 U/mL	100 U/mL	—	—
Streptomycin	100 µg/mL	100 µg/mL	100 µg/mL	—	—
Geneticin®	—	500 µg/mL	—	—	—
Recovery™ Cell Culture Freezing Medium	—	—	—	—	100%

Assay Performance with Variable Cell Number

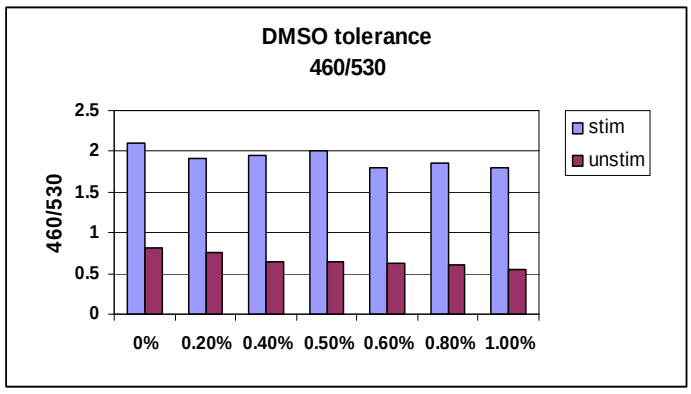
Figure 4 – PPAR gamma-UAS-*bla* HEK 293H response to Rosiglitazone with 20, 25, 30, 35, and 40K cells per well



PPAR gamma-UAS-*bla* HEK 293H cells were serum starved for 24 hours and then plated at 20,000, 25,000, 30,000, 35,000 or 40,000 cells/well in a 384-well format the day of the assay in 0.5%DMSO. Cells were stimulated with 5 μM Rosiglitazone for 16 hours, or left unstimulated. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate (1μM final concentration of CCF4-AM) for 2 hours. Fluorescence emission values at 460 nm and 530 nm for the various cell numbers were obtained using a standard fluorescence plate reader and the Ratios plotted.

Assay Performance with variable DMSO concentration

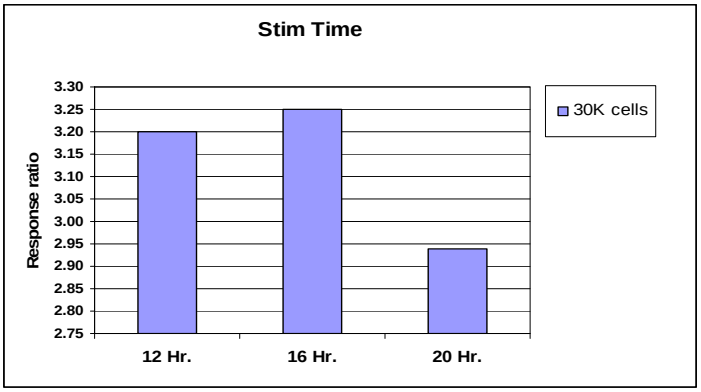
Figure 5 – PPAR gamma-UAS-*bla* HEK 293H response to Rosiglitazone with 0 - 1% DMSO.



PPAR gamma-UAS-*bla* HEK 293H cells were serum starved for 24 hours and then plated (30,000 cells/well) in a 384-well black-walled tissue culture assay plate. DMSO was added to the assay at concentrations from 0% to 1%. Cells were stimulated with 5 μM Rosiglitazone for 16 hrs, or left unstimulated. Cells were then loaded for 2 hours with LiveBLAzer™-FRET B/G Substrate (1μM final concentration of CCF4-AM). Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the Ratios are shown for each DMSO concentration.

Assay Performance with variable stimulation time

Figure 6 – PPAR gamma-UAS-*bla* HEK 293H response to Rosiglitazone with 12, 16, and 20 hour stimulation times



PPAR gamma-UAS-*bla* HEK 293H cells were serum starved for 24 hours and then plated (30,000 cells/well) the day of the assay in a 384-well black-walled tissue culture assay plate in 0.5% DMSO. Cells were stimulated with 5 μM Rosiglitazone for 12, 16, and 20 hours and then loaded for 2 hours with LiveBLAzer™-FRET B/G Substrate (1μM final concentration of CCF4-AM). Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the Response Ratios plotted.